Comparison of two commercially available toothpastes on the salivary streptococcus mutans count in urban preschool children - *an in vivo study*

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Abstract

Background & Objectives: Toothpastes have been manufactured throughout the ages all over the world in different forms as a home care measure to control dental caries. Dental caries is a multifactorial disease, the bacterial count being one of the parameters in the initiation of dental caries. Various products have been used in the past, and the present study was conducted to evaluate the efficacy of two commercially available toothpastes and their effect on the salivary streptococcus mutans count level. One was a toothpaste containing Neem, with no added fluorides, and the other a fluoridated toothpaste containing 458 ppm of fluoride. The present study was conducted 1) To assess the anti-microbial effect of two commercially available toothpastes, 2) To compare and evaluate the effect of the two toothpastes on Streptococcus mutans count level in 4 - 6 years age children with dmft 0. **Methods:** 100 student participants in the age group of 4-6 years with dmf = 0 were selected from the R.V. Public school, Bangalore. A Baseline saliva sample was taken and cultured for mutans streptococci, the colonies counted and noted. The 100 participants were randomly divided into two groups with 50 participants in each group. Group I was provided with ***Himalaya Herbals Dental cream*** containing Neem, Group II was provided with 458 ppm containing fluoride toothpaste ***Cheerio gel.*** The toothpastes and toothbrush were provided over a period of 5 months for home care use. Instructions were given to the parents on brushing technique, amount of paste to be used and twice daily brushing with the pastes, with specific instructions that the child should not use other toothpastes during these 5 months. The saliva sample was then collected and streptococcus mutans estimated in 4 phases, 1) 15 days 2) 30 days 3) 90 days 4) 150 days. **Results:** There was a steady decrease in the bacterial count over a period of 5 months; the overall percentage decrease in Group I being 90.69% and Group II being 89.69%. However, between the toothpastes there was no statistically significant difference in the bacterial count and both the toothpastes were efficacious in reducing the bacterial count. Hence it was concluded that both Herbal toothpaste containing Neem and Fluoridated toothpastes are efficacious in combating caries and both can be used as a regular home care preventive measure in combating caries in children. **Interpretation & Conclusion:** Toothpastes form a major part of the home care prevention measure in combating dental caries. Both the toothpastes have a good antimicrobial effect on caries producing salivary streptococcus mutans bacteria. Toothpaste containing Neem as well as fluoridated toothpaste are equally efficacious against caries producing bacteria.

Keywords: *Streptococcus mutans; MSB agar; Neem toothpaste; Fluoride toothpaste.*

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Introduction

Dental caries is a localized and transmissible pathological infectious process that results in the destruction of hard enamel tissue (Loesche 1986). Streptococcus mutans, an acidogenic and aciduric microorganism colonizing the oral cavity, is considered to be the main cause of dental caries (Loesche 1986, Langet al, 1987, Beighton et al, 1989). Fluoride therapy has been the cornerstone of caries-preventive strategies since the introduction of water fluoridation schemes over five decades ago. Toothpaste is

Table 1
Comparison of Baseline streptococcus mutans counts.

Baseline	N	Mean SM counts	Std. Deviation	t	P-value
Group 1	50	53720	30495.9	0.901	0.37
Group 2	50	48520	27099.4		

The Baseline mean bacterial count in Group 1 is 53720 and Group 2 is 48720. From the above table we notice that there is no significant difference between Group 1 and Group 2 in Baseline bacterial counts (P>0.05).

by far the most widespread form of fluoride usage (Murray 1991a; Ripa 1991) and the decline in the prevalence of dental caries in developed countries is mainly attributed to its increased use (Glass 1982; Rolla 1991; Marthaler 1994; O'Mullane 1995; Marthaler 1996).[1] The intensive promotion of fluoridated toothpastes by the oral health care Industry is a major factor in their increased use. Since the 1980s nearly all commercially available toothpaste formulations contain fluoride.[2] However, Neem (Azadirachta indica) has been used in India and South Asia for thousands of years to clean the teeth and fight bacterial and fungal infections. Modern science validates that Neem has antimicrobial properties. Studies suggest that Neem extract is appropriate for treating gingivitis and oral infections as it inhibits the formation of plaque and the growth of bacteria.[3] As the popularity of natural medicines and dentifrices continues to rise, dental professionals are in a position to provide information to patients about these products' safety and efficacy. This can be difficult, owing to a lack of professional consensus on the subject. To date, an insufficient amount of clinical research on herbal-based dentifrices has been reported, in contrast with a plethora of such research for conventional flouridated oral care products. A lack of scientific studies on natural and herbal products in the peer- reviewed dental literature poses a conundrum for health care professionals when dealing with these products. While some of these natural products could be just as safe as conventional dentifrices, others may pose various risks if used incorrectly. In addition, as only a limited number of in vivo studies on herbal dentifrices have been published, it has not been determined whether they are superior, equivalent or substandard to conventional dentifrices in reducing plaque, for example. Furthermore, few research efforts are directed toward addressing the potency or quality of herbal ingredients used in these dental products. While many herbal toothpastes claim to have antimicrobial properties, very little research was conducted to investigate these claims.[4] This study therefore seeks to evaluate the efficacy of fluoride and Neem containing toothpastes which are commercially available (over-the-counter) on the salivary Streptococcus mutans count level as well as to compare if any difference exists in the antimicrobial action between the fluoridated and Neem containing toothpastes or both have equal efficacy against salivary streptococcus mutans.

Objectives

1) To assess the anti-microbial effect of two commercially available toothpastes on salivary Streptococcus mutans.
2) To compare and evaluate the effect of the two toothpastes on salivary Streptococcus mutans count level in 4 - 6 years age children with dmft 0.

Methodology

The study was conducted on preschool children in the age group of 4-6 years in Bangalore city. 100 children of both sexes were selected from R. V. PUBLIC school (males 60 & females 40). Written informed consent was obtained prior to the enrolment from the parents of all the students participating in the study. General data was collected from the parent(s) during a visit to the school using the BASIC QUESTIONAIRE FOR INTERVIEWING MOTHERS by Poul Eric Petersen, 2003 (WHO). A total of 100 children in the age group of 4-6 years from the R.V. PUBLIC school who fulfilled the inclusion and the exclusion criteria were selected to participate in the study.
The Inclusion criteria for selection: 1. DMFT=0
The Exclusion criteria for selection :
1. Marked intra oral soft tissue pathology.
2. Subjects with history of taking antibiotics three months prior or during the course of study.
3. Medically compromised patients
4. Children undergoing orthodontic therapy.
5. Children with history of professionally applied topical fluoride.

Table 2

Comparative values of Streptococcus mutans count on the 15th day after tooth paste therapy in each group.

Time	Group	N	Mean SM Counts	Std. Deviation	T	P-value
15th Day	Group 1	50	28640	8918.6	0.404	0.687
	Group 2	50	27780	12144.2		

Group 1 shows a mean bacterial count of 28640, and Group 2 shows a mean bacterial count of 27780. From the above table we notice that there is no significant difference between Group 1 and Group 2 on 15th day bacterial counts (P>0.05).

Table 3

Comparative values of streptococcus mutans count on the 30th day after tooth paste therapy in each group

Time	Group	N	Mean SM Counts	Std. Deviation	T	P-value
30th Day	Group 1	50	19640	8191.0	0.135	0.893
	Group 2	50	19420	8139.3		

Group 1 shows a mean bacterial count of 19640, and Group 2 shows a mean bacterial count of 19420. From the above table we notice that there is no significant difference between group 1 and group 2 in 30th day bacterial counts (P>0.05).

The study took place over a period of 5 months from February 2007 to July 2007. The WHO proforma 1997 dentition status assessment was used to calculate DMF. Saliva collection (to assess the microbial count) was done at the period of Baseline, 15 days, 30 days, 90 days and 150 days. A washout period of 2 weeks was given prior to the Baseline count wherein the children brushed their teeth with their regular brush, but without a toothpaste.

Saliva collection: Three students at a time were made to sit comfortably on the chair. After swallowing pre-existing saliva, subjects were given paraffin wax to chew to stimulate salivary flow which was then collected by expectorating in a sterile disposable measuring cup over the next 5 minutes.

Standardisation of the saliva collection technique: ***1.*** The subject did not eat or drink (except water) 1 to 2 hours before collection. ***2.*** The subject did not perform any physical exercise before collection. ***3.*** The saliva was collected over a period of 5 minutes. ***4.*** A standardized sterile paraffin block supplied by the ***Thomas Baker (Chemicals)*** was used as the stimulant. By means of a sterile disposable syringe, a 1ml aliquot of saliva was removed from the measuring cup and injected in a previously labeled sterile bottle containing 4 ml of transport medium (Thyoglycollate broth). The inoculated bottles were transported to laboratory immediately within an hour. The processing was done in the Dept of Microbiology D.A.Pandu Memorial.R.V. Dental College, Bangalore.

Laboratory procedures

The sample was vortexed to uniformly mix the saliva and media using a Cyclo mixer. Using an inoculation loop (standard loop with 4mm diameter) 10µL of the vortexed sample was streaked in duplicate on Mitis Salivarius Bacitracin agar selective for Streptococcus mutans. The mitis salivarius agar plates were incubated in aerobic conditions for 48hrs at 37 degree Celsius in an incubator.The plates were opened after 48 hrs. The counts were made of colonies with morphologic characteristics of streptococcus mutans (0.5 mm raised convex undulated colonies of light blue color with rough margins, granular frosted glass appearance)[4] on the plates using a magnifying lens and were expressed as number of colony forming units (CFU) per/ml of saliva (Figure 1). Semiquantitation of the number of colonies was done by multiplying the actual colony count with 1×10^3 as the sample was diluted one thousand times (1:5 dilution). The Baseline scores were noted for all the 100 children. The children were then randomly assigned to one of the toothpastes under evaluation: 1) Himalaya Herbals Dental cream (The Himalaya Drug company). 2) Cheerio gel (Dr Reddy's Laboratories Ltd). The toothbrushing technique (Fonne's Technique) was demonstrated to the parents. The

Table 4

Comparative values of streptococcus mutans count on the 90th day after tooth paste therapy in each group.

Time	Group	N	Mean SM Counts	Std. Deviation	t	P-value
90th Day	Group 1	44	10200	6090.5	-0.206	0.838
	Group 2	39	10490	6984.5		

Group 1 shows a mean bacterial count of 10200, and Group 2 shows a mean bacterial count of 10490. From the above table we notice that there is no significant difference between group 1 and group 2 in 90th day bacterial counts (P>0.05).

Table 5

Comparative values of streptococcus mutans count on the 150th day after tooth paste therapy in each group.

Time	Group	N	Mean SM Counts	Std. Deviation	T	P-value
150th Day	Group 1	44	5750	4420.5	0.766	0.446
	Group 2	39	5000	4483.9		

From the above table we notice that there is no significant difference between group 1 and group 2 in 150th day bacterial counts (P>0.05).

toothpastes and Oral-B Kid toothbrush was distributed to the children. The parents were asked to place an amount of toothpaste equal to the size of a pea on the tooth brush. The children were then asked to brush twice daily with the toothpastes for 5 months. The parents were asked to monitor the child's brushing twice a day for 5 months. Saliva samples were then collected at the end of 15 days, 30 days, 90 days and 150 days. The samples were cultured and colonies of Streptococcus mutans counted and compared. Gram staining was done to confirm the growth of Streptococcus mutans.

Results

A Total of 100 subjects participated in the study. 35 were 4 year old, 32 were 5 year old , 33 were of 6 years old.

In Group I - Himalaya Herbal Dental cream: 18 subjects were 4 years old, 16 were 5 years old and 16 were 6 years old.

In Group II: Cheerio Gel: 17 were 4 years old,16 were 5 years old and 17 were 6 years old. Out of the 100 subjects, 60 were male and 40 were female subjects.

Baseline Streptococcus mutans count.

At the Baseline Group I had a mean Streptococcus mutans count of 53720 counts/ml. Group II had 48520 counts/ml. When a t-test was performed, the p- value was 0.37, which was not significant. Hence it is inferred that the difference in the mean bacterial count between the two groups at Baseline was not statistically significant. (Table 1).

After 15 days of Toothpaste Therapy – Group I had a mean bacterial count of 28640 counts/ml and Group II had a mean bacterial count of 27780 counts/ml. When a t-test was performed the p-value was 0.687. The percentage reduction in Group I was 46.69% and Group II was 42.75%. Although there has been a significant reduction of the bacterial count in the two groups when compared to the Baseline, there is no statistically significant difference between the two toothpastes. (Table 2).

After 30 days of Toothpaste Therapy, Group I had a mean bacterial count of 19640 counts/ml and Group II a mean bacterial count of 19420 counts/ml. When a t-test was performed, the p-value was 0.893. The percentage reduction in Group I was 63.44% and Group II was 59.98% when compared to the Baseline. Although there has been a significant reduction of the bacterial count in the two groups when compared to the Baseline, there is no statistically significant difference between the two toothpastes. (Table 3). The number of children after 90 days were 83 due to a change of school.

After 90 days of Toothpaste Therapy, Group I had a mean bacterial count of 10200 counts/ml and Group II a mean bacterial count of 10490 counts/ml. When a t-test was performed, the p-value was 0.838. The percentage

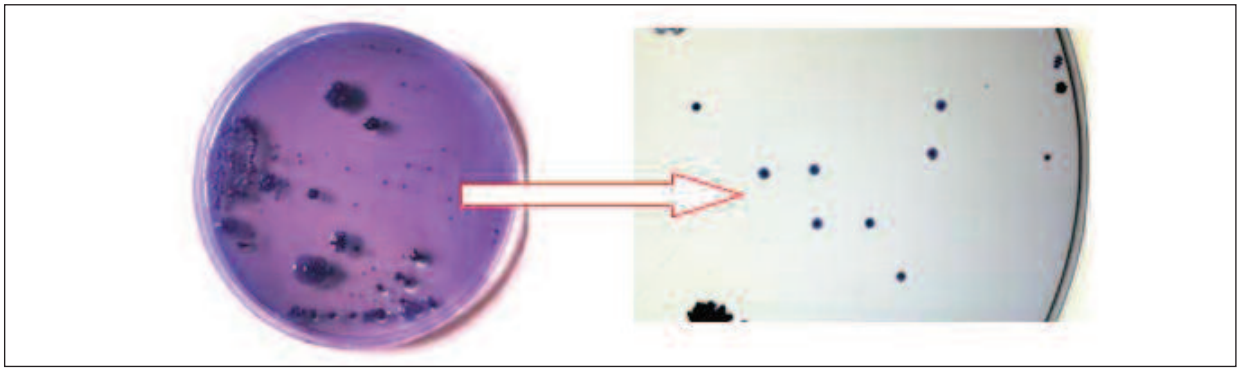

Figure 1: Streptococcus mutans colonies on Mitis Salivarius Bacitracin Agar.

reduction in Group I was 81.02 % and Group II was 78.39 % when compared to the Baseline. Although there has been a significant reduction of the bacterial count in the two groups when compared to the Baseline, there is no statistically significant difference between the two toothpastes. (Table 4).

After 150 days of Toothpaste Therapy, Group I had a mean bacterial count of 5750 counts/ml and Group II had a mean bacterial count of 5000 counts/ml. When a t-test was performed, the p-value was 0.446. The percentage reduction in Group I was 90.69 % and Group II was 89.69 % when compared to the Baseline. Although there has been a significant reduction of the bacterial count in the two groups when compared to the Baseline, there is no statistically significant difference between the two toothpastes. (Table 5).The overall percentage change in the bacterial counts from Baseline in Group I was 90.69% and Group II was 89.69%.

Discussion

Use of fluorides has been the cornerstone of caries prevention programmes and the use of fluoridated toothpaste is by far the most common form of caries control in use today.[1,5] The advantages of fluoridated products is well documented in the literature. It is the most spoken and debated topic all over the world with researchers having diverse views about its use and safety.[6] The caries preventive effect of low fluoride toothpastes may be slightly less than that of adult strength alternatives but considering the fact that most children swallow toothpaste while brushing and the adverse effects associated with it, the use of low-fluoride toothpaste by children under 6 years of age seems warranted. However, Neem has been used in India and South Asia for thousands of years to clean the teeth and fight bacterial and fungal infections. Modern science validates that Neem has antimicrobial properties. With available modern preparations many people are now using commercial products that contain the same basic Neem components. A lack of scientific studies on natural and herbal products in the peer-reviewed dental literature poses a conundrum for health care professionals when dealing with these products. Furthermore, few research efforts have been directed toward addressing the potency or quality of herbal ingredients used in these dental products. It is generally agreed that, with the exclusion of fluoride from most natural herbal dentifrices, these dentifrices usually forfeit caries-preventive benefits. While many herbal dentifrices claim to have antimicrobial properties and have caries preventive action, very little research has been conducted to investigate these claims.[7] Hence the purpose of this study was to compare and evaluate the efficacy of fluoridated toothpaste (containing 458 ppm of fluoride) and Neem toothpaste (which are commercially available) on the salivary streptococcus mutans count level in urban pre-school children aged 4-6 years with dmf=0 when used as a preventive oral home care measure. A highly significant correlation has been demonstrated between the salivary numbers of mutans streptococci and the prevalence of dental caries in the dentition, both in terms of the number of tooth surfaces colonized and the level of infection of tooth surfaces (Duchin and van Houte, 1978; Lindquist, 1991). Hence saliva sample was taken for this study which was easy to obtain from children by chewing paraffin wax for 2-5 minutes[8] .In the present study, the study group under evaluation consisted of 100 subjects who met the inclusion/exclusion criteria. The Baseline bacterial count were taken. The subjects were randomly allocated to the two toothpastes under evaluation consisting of 50 children in each group. The subjects were asked to brush twice daily with an Oral–B Kid tooth brush which was provided to them. They were asked to place a pea size amount of toothpaste on the brush under parental supervision. This is in accordance with the FDI statement

accepted in November 2000 in Paris.[7] The sample was repeated after 15 days, 30 days, 90 days and 150 days of toothpaste therapy and cultured for Streptococcus mutans. Only 83 students remained at the end of the study. In the present study there was no significant difference between the groups with respect to the total number of salivary streptococcus mutans at Baseline. Group 1 had mean bacterial count of 53720 CFU/ml and Group 2 had a mean bacterial count of 48520 CFU/ml with a p-value of 0.37, which was not significant. This can be explained by the fact that there was not much difference among the group at Baseline as the dmf (decay component) was standardized with dmf =0 [9] After 15 days of toothpaste therapy there was a 46.69% reduction in group I using Neem paste and 42.75% reduction in the group using fluoride toothpaste when compared to the Baseline values [3,4,24] In the present study, a steady decrease in the mean bacterial count has been observed both in the group using the Neem toothpaste as well as the fluoridated toothpaste, both the toothpastes have effectively reduced the salivary Streptococcus mutans count when used over a period of 5 months. This substantiates the antibacterial properties of the toothpastes. This is similar to the results seen in the previous studies on both the Fluoridated toothpastes as well as Neem toothpaste. Both the toothpastes have antimicrobial properties against the Streptococcus mutans bacteria.[3,4,10] This study correlates with the study[4] in which the participants were given fluoride products containing 500ppm and 1000ppm of fluoride, where both showed significant reductions of up to 50% after 21 days which is similar to the results obtained in the present study. However the study was conducted for 21 days and no follow up was done to the effects after long term use. There was a significant reduction seen in bacterial count after 30 days with a reduction of 63.44% in Neem containing toothpastes and 59.98% reduction in fluoride containing pastes compared with the Baseline count. There was also a significant reduction of 81.02% & 78.39% in Neem toothpaste and fluoride toothpaste respectively after 90 days. At the end of 150 days, a total reduction of 90.69% was seen with the Neem toothpaste and 89.69% with the fluoride toothpaste.[11-14]

Studies of Glass 1982, Ripa 1999, Rolla1991, Marthaler 1994, Brathall 1996 have attributed the gradual decrease in caries to the regular home care use of fluoride in toothpaste, however this continues to be debated. Contradicting this study is one in which the researchers found no difference in the plaque ecology using fluoridated toothpaste.[15] This study used a very small sample size of 7 subjects and the time interval was 5 days. An in-vitro study conducted on albino rats comparing a regular fluoridated toothpaste and herbal toothpaste has shown a reduction of 54.37 % with herbal toothpaste and 37.86% with fluoride toothpaste. The trial lasted for 21 days. The results of this study are similar to the present study, however 21 days was a short duration for the assessment. Triclosan has been added to fluoridated toothpastes for its anti bacterial action. However in this particular study,[15] the addition of triclosan to the conventional fluoride showed no statistically significant difference. Studies (Charter 992, O'Mullane 1997, Chestnut 1998, Ashley 1999) show that the habits of children and adults using fluoride toothpaste, for instance the frequency of use, influence effectiveness. These studies indicate that brushing twice daily with a fluoride toothpaste confers greater caries reduction than brushing once daily or less. This correlates to the present study where children improved their oral hygiene measures along with the frequency and good brushing technique which has obtained good bacterial results.[16-18] Neem has known anti-bacterial properties which explains its sustained action after 150 days of use. This is in correlation with the study wherein a steady decrease in streptococcus mutans count has been observed after the use of Neem paste.[3] In a study conducted to check the inhibitory zones to streptococci, it was shown that water soluble extracts of the Neem stick extracts affect some bacterial properties which may alter bacterial adhesion and the ability of some streptococci to colonize tooth surfaces.[19] In another study on antimicrobial action of 7 Asian chewing sticks, there was no inhibition zone seen with streptococcus mutans by Neem extract at 50% concentration. The reason may be due to the low concentration used as well as the fact that the study took one month and extracts were not fresh at the time of the study.[20-23] However, in the present study, there has been no statistically significant difference in the performance between the two toothpastes, and both the toothpastes have been efficacious in reducing the caries causing bacteria. The anti-caries activity of both toothpastes can be analysed only after a good follow up of the study. Although the bacterial count has shown reductions over a period of 5 months, the prolonged effect on the bacterial count needs to be checked as well as whether the reduced count is stabilized or the counts return back to Baseline values. Whether there is any bacterial resistance developing to these products also needs to be monitored carefully. Maintainence of good oral hygiene cannot be over-emphasized, hence good oral hygiene with twice brushing and efficient technique is the cornerstone of

caries prevention, no matter which product is used .The Neem toothpaste as well as fluoridated toothpaste with fluoride content of 458ppm are effective and safe as part of the Daily home care measures for caries prevention in children.[3,4,10,24]

Conclusion

The present study was conducted to compare two commercially available toothpastes and their effect on salivary Streptococcus mutans count level in urban preschool children. The two toothpastes under evaluation were: Group I, Himalaya herbal dental cream containing Neem as the basic component and Group II, Cheerio gel having fluoride as its basic component. From the present study the following conclusions can be drawn:

1) The two toothpastes under evaluation have shown a significant reduction in the salivary Streptococcus mutans count when compared to the Baseline values. Hence it can be concluded that both the toothpastes have a good antimicrobial effect on caries producing salivary streptococcus mutans bacteria.

2) The toothpastes under evaluation have been effective at all the time intervals. However, when compared with each other, there is no significant difference in their performance. Hence it can be concluded that both Neem containing toothpaste as well as a fluoridated toothpaste are equally efficacious against dental caries initiating Streptococcus mutans bacteria and can be used in children as a regular home care preventive aid in combating dental caries. In the present study, the toothpastes were evaluated for caries initiating bacteria i.e., Streptococcus mutans. However, further studies are needed to know the efficacy of these toothpastes on Lactobacillus, the bacteria responsible for the progression of Dental Caries. The advantages of Fluoride is well documented, however, there is not much research work undertaken on the naturally and easily available Neem (Azadiracta Indica). Further research has to be undertaken to extract the advantages of Neem (Azadiracta Indica) which is so easily available in India.

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